SECTION III

OPERATIONAL SYSTEM CONSIDERATIONS FOR HARDWARE STERILIZATION

N70-73642

A. SCHEDULING OF STERILIZATION OPERATIONS

1. The systems approach to scheduling of sterilization operations yields activities which involve several disciplines. Figure 3-1 is a schedule which shows the interrelationships and dependencies between activities; it was developed from activities listed in paragraph B of this section and from a Voyager schedule by G. Robillard dated 25 February 1965. The events from the Voyager schedule were included to inject some realism; however, Figure 3-1 is not to be interpreted as anything but an example of the interrelationships between sterilization activities and the overall program.

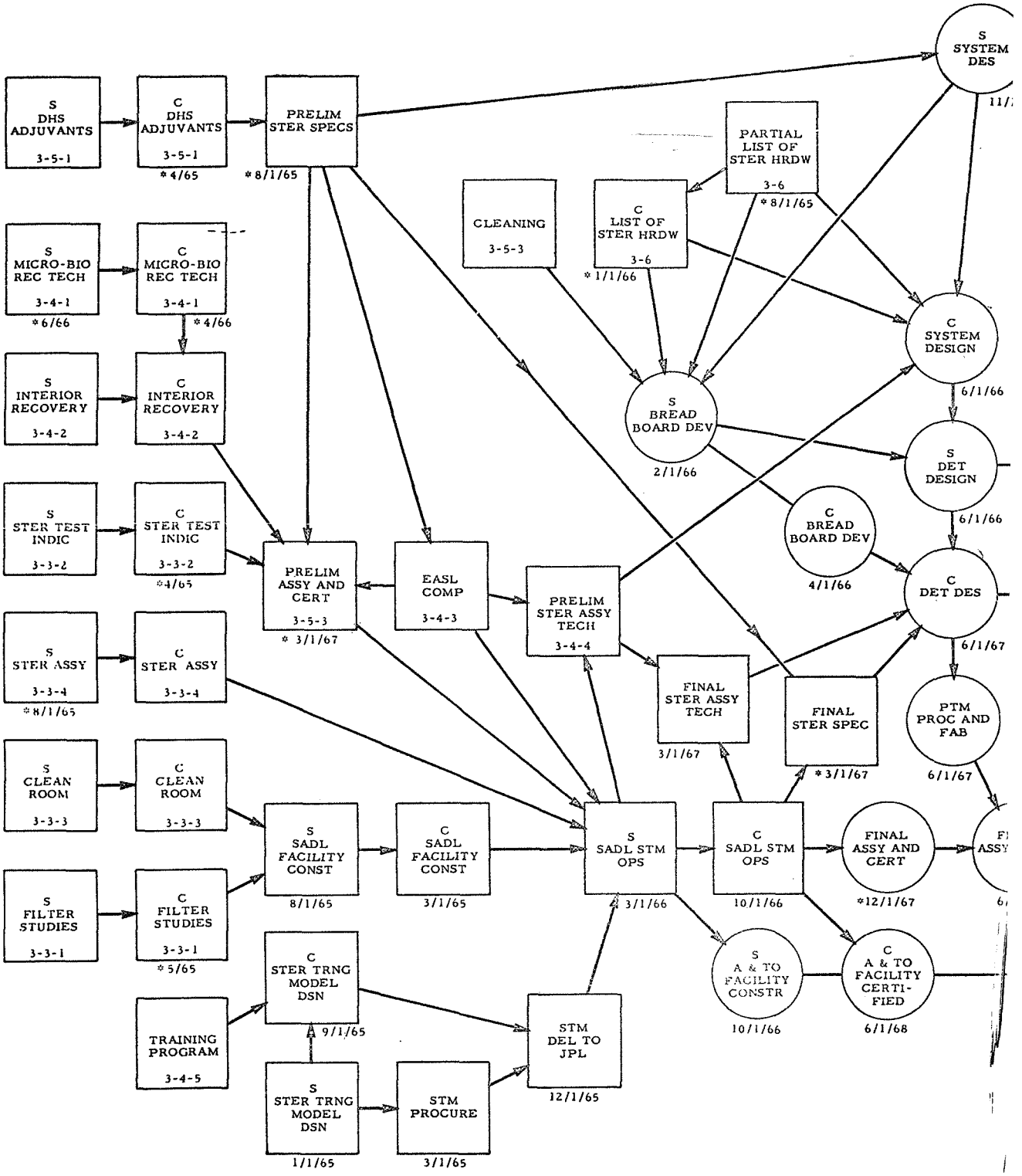
2. Figure 3-1 is an example of scheduling for sterilization activities.

a. Coding for Figure 3-1:

1) The square events are a direct result of the sterilization; i.e., if there were no sterilization requirements, the squares would be removed from the schedule.

2) The circle events would be standard for a program not requiring sterilization. This is not to infer that the details of circle events would be unchanged with or without sterilization; however, they would, in title and intent, remain unchanged.

3) The code in the lower part of the event symbol is a reference to the appropriate activity listed in Tables 3-1 through 3-6; e.g., 3-3-1 is the activity on filter studies outlined in Table 3-3, No. 1.


S DHS ADJUVANTS 3-5-1
C DHS ADJUVANTS 3-5-1
*4/65
PRELIM STER SPECS
*8/1/65
S SYSTEM DES
11/
PARTIAL LIST OF STER HRDW 3-6
*8/1/65
CLEANING 3-5-3
C LIST OF STER HRDW 3-6
*1/1/66
S MICRO-BIO REC TECH 3-4-1
*6/66
C MICRO-BIO REC TECH 3-4-1
*4/66
C SYSTEM DESIGN
6/1/66
S INTERIOR RECOVERY 3-4-2
C INTERIOR RECOVERY 3-4-2
S BREAD BOARD DEV
2/1/66
S DET DESIGN
6/1/66
S STER TEST INDIC 3-3-2
C STER TEST INDIC 3-3-2
*4/65
C BREAD BOARD DEV
4/1/66
PRELIM ASSY AND CERT 3-5-3
* 3/1/67
EASL COMP 3-4-3
PRELIM STER ASSY TECH 3-4-4
C DET DES
6/1/67
S STER ASSY 3-3-4
*8/1/65
C STER ASSY 3-3-4
FINAL STER ASSY TECH
3/1/67
FINAL STER SPEC
*3/1/67
PTM PROG AND FAB
6/1/67
S CLEAN ROOM 3-3-3
C CLEAN ROOM 3-3-3
S SADL FACILITY CONST
8/1/65
C SADL FACILITY CONST
3/1/65
S SADL STM OPS
3/1/66
C SADL STM OPS
10/1/66
FINAL ASSY AND CERT
*12/1/67
S FILTER STUDIES 3-3-1
C FILTER STUDIES 3-3-1
*5/65
C STER TRNG MODEL DSN
9/1/65
S A & TO FACILITY CONSTR
10/1/66
C A & TO FACILITY CERTI-FIED
6/1/68
TRAINING PROGRAM 3-4-5
STM DEL TO JPL
12/1/65
S STER TRNG MODEL DSN
1/1/65
STM PROCURE
3/1/65

NOTES: 1. ALL DATES PRECEDED BY AN ASTERISK ARE ESTIMATED.
2. ALL OTHER DATES ARE AS SHOWN ON VOYAGER CAPSULE SCHEDULE (SEE PAR III. A. 1.)

□ EVENTS PECULIAR TO THIS PROGRAM DUE TO THE STERILIZATION REQUIREMENT.

○ EVENTS NORMAL TO PROGRAM, IF STER REQUIREMENT REMOVED

— SEE PAR III. A. 2

S = START
C = COMPLETE

3. THE CODE IN THE LOWER PART OF THE EVENT SYMBOL REFERS TO THE APPROPRIATE ACTIVITY LISTED IN TABLES 3-1 THROUGH 3-6; e.g., 3-3-1 IS THE ACTIVITY ON FILTER STUDIES DESCRIBED IN TABLE 3-3, NO. 1

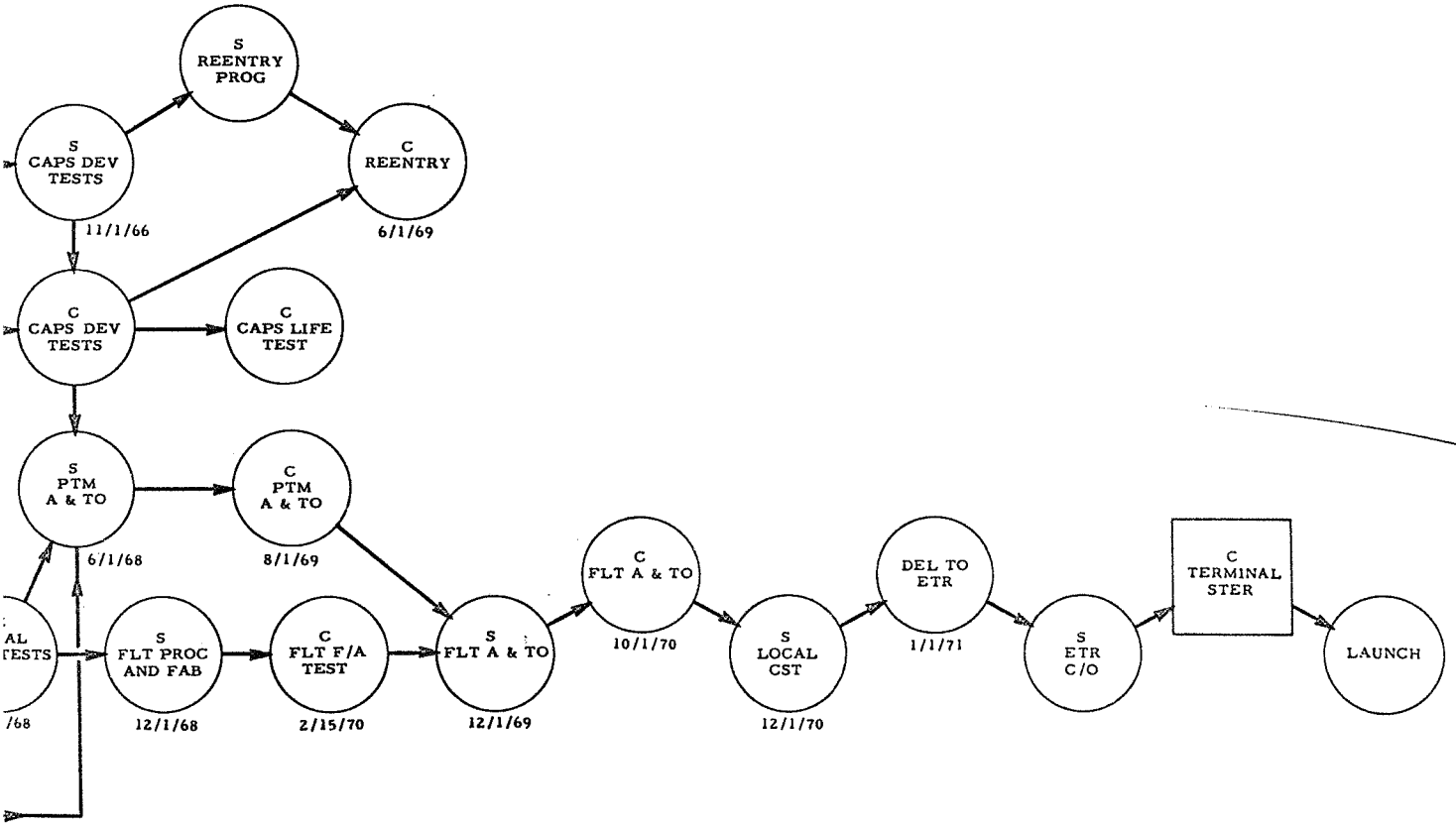


Figure 3-1. Typical Assembly Sched

B. REVIEW AND RECAPITULATION OF MICROBIOLOGICAL RESEARCH ACTIVITIES TO IMPLEMENT STERILIZATION

The Planetary Quarantine Program of the Bioscience Programs Office of NASA is sponsoring a number of tasks concerned with sterilization. Tables 3-1 through 3-6 describe the major studies that have been completed, are in progress, or are scheduled for Fiscal Year 1966.

Table 3-1. Completed Sterilization Studies

Number	Subject	Description
1	Electronic parts	A series of tests were conducted to assay electronic parts for viable microorganisms. Resistors, transistors, and capacitors were found to have internal bacterial contamination in up to 20 to 25 percent of the parts tested. Moreover, viable microorganisms were recovered from the interiors of some parts that had been heated at 125°C for 13.5 hr. Exposure to an ethylene oxide/Freon 12 mixture (ETO-F 12) did not sterilize components internally inoculated with bacterial spores. However, viable spores were not recovered from the exterior surfaces of any of the inoculated parts after exposure to ETO-F 12 for 6 hr. Another test was conducted to determine if electronic parts could be sterilized by dry heat (125°C for 27 hr). The results obtained were inconclusive, for viable microorganisms were not recovered from the interiors of <u>any</u> of the resistors, capacitors, cores or chokes tested, whether heated or unheated. These data indicated a need for more definitive work and NASA has funded an expanded study to be conducted by the Jet Propulsion Laboratory. A later report in the Detrick series tabulated the physical composition, method of assembly, resistance to heat and radiation, and potential for bacterial contamination, of various electronic parts that may be used in or on spacecraft which are required to be sterile.
2	Solar panel, balsa wood, pyrotechnics, and propellants	Results indicated that the solar panel tested had internal contamination that could not be sterilized by exposure to ETO for 6 hr. Microbial contamination was also present in each type of explosive charge tested (squibs, pyrotechnic pellets) and in the samples of finished propellant that were assayed.
3	Dry heat sterilization	Mixtures of bacterial spores and diatomaceous Earth were heated at several temperatures through several different exposure periods. Heating at 105°C for 36 hr or at 125°C for 6 hr did not sterilize the mixture. Continued heating at 125°C for 12 hr appeared to reduce the number of viable spores in the mixtures. These data indicated a need for considerably more work in this area and NASA has sponsored an expanded effort by the Wilmot Castle Company. The Castle study was spread over a 3-yr period and a considerable amount of information was obtained.

Table 3-1. Completed Sterilization Studies (contd)

Number	Subject	Description
4	Ethylene oxide and radiation	A motor was internally and externally inoculated with bacterial spores. External, but not internal surfaces, of the contaminated motor could be decontaminated with ETO (6 hr exposure period). Radiation, in the dosage used (270-27,000 Roentgens), had no effect on dry bacterial spores.
5	Sporocides, resins, and batteries	Of some 5 resins tested, Epon 828 with Epocast 985 and ethylene amine in absolute methanol appeared to be the most promising selfsterilizing resin. However, after several tests, the Fort Detrick group did not anticipate that many of the resins would possess selfsterilizing properties in themselves, but that many resins might have selfsterilizing activity when a sporocidal compound was incorporated with the monomers. A nickel-cadmium battery was tested for its selfsterilizing properties. Several tests were made to determine if bacterial spores would survive in parts of the battery. All results indicated the type of nickel-cadmium battery tested was selfsterilizing.
6	Ultrahigh vacuum	Three species of microorganisms were exposed for 5 days to an ultrahigh vacuum approaching that of interplanetary space. Since no lethal effect was observed, there was no indication that the vacuum of outer space would prevent transport of viable microorganisms on contaminated spacecraft.
7	Filtration	The filtering efficiency of several types of filters was checked with an aerosolized suspension of bacterial spores (1 to 5 μ diameter). Results indicate that the Robbins-type filter using unbonded fiberglass was quite effective in removing bacterial spores from an airstream. A second series of filtration experiments were conducted to determine the number of viable microorganisms in nitrogen gas stored in cylinders (6340 liters of gas compressed to 2000 psi). Results showed an average of 1 microorganism per 33 cu ft of unfiltered nitrogen. Filtration of the nitrogen would drastically reduce this level of contamination.

Table 3-1. Completed Sterilization Studies (contd)

Number	Subject	Description
8	Room air surface contamination	Sterile stainless steel strips (1 by 2 in.) were exposed to room air for various time periods ranging from 1 to 103 days. Half of the strips were placed in a horizontal position on a clean decontaminated shelf; the rest were placed in a vertical position on the same shelf. Also, human volunteers briefly handled or touched both sterile stainless steel strips and sterile electronic components (resistors) with their fingers. Microbial recovery from stainless steel strips indicated that more viable microorganisms from the air fall on a horizontal surface than on a vertical surface. Over a 3-month period the microbial population on the steel, positioned either horizontally or vertically, seemed to build up to a certain level and then remain fairly constant. The results obtained after surfaces were touched or handled by clean room workers showed that the number of microorganisms recovered from stainless steel strips and electronic components varied from day to day for any one volunteer as well as among the volunteers. The person, rather than the type of surface, probably determines the amount of microbial contamination present after handling. Sixty-four percent of the volunteers left very few microorganisms upon handling the steel, while 8 percent of the volunteers greatly contaminated the steel. In a second test similar to that described above, approximately 1000 microorganisms were recovered from a stainless steel strip (approximately 500/sq in.) after exposure to room air for 33 days. None of the stainless steel strips exposed to 135°C for 12 hr or for 24 hr contained any viable microorganisms as evidenced by the lack of microbial growth after 7-days incubation in one type of cultural medium.

Table 3-1. Completed Sterilization Studies (contd)

Number	Subject	Description
9	Cleanroom surface contamination	Sampling was done in a factory area and also within a cleanroom located in one corner of the factory area. The data collected during an 11-wk period indicated that about 10 times more microorganisms per cubic foot of air were in the factory than in the cleanroom, and aerobes seem to be about 4 times more prominent than anaerobes. This ratio was also noted in the data showing the rate that airborne microorganisms settle onto a surface. From the data, there is an indication that some microorganisms recovered from the cleanroom survived heat shocking. It is seen that aerobes were more prominent than anaerobes. These results were based upon very few microorganisms (from 3 to less than 1 per stainless steel strip) and, therefore, are not adequate to establish a reliable ratio. The data collected also indicated that the microbial population on stainless steel remainted fairly constant throughout the 11-wk test period. The microbial population may be increased if the surface is touched frequently by the human hand as would be the situation during the construction of a spacecraft. The data collected during the first 11-wk period of this study appear to indicate that microbial contamination is reduced about 10-fold in the cleanroom samples. A second test was conducted to determine the microbial contamination that results in cleanrooms occupied by operating personnel. The bacteriological results indicate that when the cleanroom was vacant, there was very little microbial contamination, but when the room was occupied with personnel the microbial contamination was greatly increased, even when a surgical mask was worn in addition to the cleanroom clothing. An additional increase in microbial contamination was noted with personnel movement. When the subjects were moving in the cleanroom in their street clothes, the level of microbial contamination again increased. However, the level of microbial contamination was rapidly reduced immediately after the personnel leave the cleanroom. The data indicated that the level of microbial contamination resulting from the presence of personnel would depend upon the restrictive measures imposed (e.g., the amount of movement, the type of clothing, or the use of masks).

Table 3-2. Planetary Quarantine Activities in Progress Not Under JPL Cognizance

No.	Subject	Contractor	Description
1	Role of metal ions in spore physiology	Syracuse University Cognizant scientist: R. Slepecky	The goal is to determine why various metal cations are required for the sporulation process in bacteria and to investigate how metals participate in the germination process of spores. The results of this investigation will lead to an understanding of why the participation of metals is a necessary requirement for sporulation. One can then devise new approaches to overcome the stability that the metals impart to the spore. Alternatively, one can gain an insight into how to induce the spore to come out of this stable state.
2	A sono-chemical approach to sterilization problems	St. John's University Cognizant scientist: M. Pisano	The purpose of this project is to improve the killing rate of gaseous ethylene oxide molecules by means of sonic excitation through which the destruction of superficial protective films or the acceleration gas diffusion occurs.
3	Evaluate practical techniques for reducing microbial dissemination from cleanroom personnel, reduction of the microbiological contamination on surfaces by intermittent low-level heating at controlled relative humidity, and the kinetics of bacterial kill by ethylene oxide.	Communicable Disease Center (CDC), United States Public Health Service (USPHS) Cognizant scientists: G. F. Mallison and P. Skaliy	The Phoenix Field Station is the forerunner of the NASA Sterility Control Laboratories (Cognizant scientists: M. Goodwin and M. Favero). Here, CDC will establish, equip, and staff the base microbiology laboratory to provide support for the NASA Headquarters SterCon Group. If a service is needed at other points in the U.S., the requirement will be provided from the Phoenix lab by short-term assignment of personnel or by the establishment of field laboratories. The Medical Audiovisual Branch of CDC will provide a training package (training courses, illustrated materials, etc.) to implement the NASA spacecraft sterilization program.

Table 3-2. Planetary Quarantine Activities in Progress
Not Under JPL Cognizance (contd)

No.	Subject	Contractor	Description
4	Kinetics of dry-heat sterilization	Massachusetts Institute of Technology Cognizant scientist: G. Silverman	The technical objectives of this study include: 1) A critical examination of physiological factors which influence microbial resistivity and which will be a source of data for the derivation of precise mathematical equations to express the kinetics of dry-heat sterilization. 2) A determination of the thermal resistance of various microorganisms, including those organisms which constitute the natural flora of various materials associated with spacecraft and their manufacture.
5	Cleanroom microbiology	University of Minnesota Cognizant scientists: G. Michaelson and D. Vesley	As part of the sterilization of lunar and planetary landers, assembly is required in cleanrooms on the assumption that this practical will reduce the microbial loading. However, very little is known of the bacteriology of cleanrooms or the best methods of operation. This study will look into some phases of the microbiology of cleanrooms to the end that restrictions on operations may be minimum while still ensuring minimum biological loading. Study goals include: 1) Determine the quantity of bacteriological contamination in representative cleanrooms. 2) Identify the more common organisms found.

Table 3-2. Planetary Quarantine Activities in Progress Not Under JPL Cognizance (contd)

No.	Subject	Contractor	Description
5	Cleanroom microbiology (contd)		3) Determine the heat resistance of the organisms encountered. 4) Determine design and procedural features of cleanrooms that will keep microbial contamination, particularly spores, to a minimum.

Table 3-3. JPL Sterilization Group Tasks in Progress

No.	Subject	Contractor	Decription
1	Microbiological filters for sterilizing gases and liquids	JPL/Wilmot Castle	A study to evaluate the efficiency and reliability of currently available filters to sterilize liquids and gases. Results obtained in this study should provide the basis for selection of filters for use in the production of sterile spacecraft. Reviewed in JPL Space Programs Summary (SPS) No. 37-29, Vol IV.
2	Biological sterility indicator for dry-heat sterilization	JPL/Wilmot Castle	The objective of dry heat sterilization of spacecraft is death of all biological life in or on the spacecraft. Therefore, it is logical to employ a biological indicator as the absolute test for sterility. Bacterial spores from resistant isolates, on different carrier media, will be subjected to dry heat sterilization. This study will result in the development of heat resistant microorganisms contained in or on a suitable carrier that will indicate sterility only when sterility has, indeed, been achieved. Reviewed in JPL SPS No. 37-29, Vol IV.
3	Microbiological profile of cleanrooms	JPL/Douglas Aircraft Co., Inc.	An extensive study to determine the levels of microbial contamination on surfaces and in the air of Class II, III, and IV cleanrooms over a 6-month period during various conditions of activity. Human handling experiments are also being conducted. Air, surface and handling samples will be assayed for viable sporogenous and asporogenous aerobic and anaerobic mesophiles, thermophiles, and fungi. Reviewed in JPL SPS No. 37-29, Vol IV.

Table 3-3. JPL Sterilization Group Tasks in Progress (contd)

No.	Subject	Contractor	Description
4	An experimental study of sterile assembly techniques	JPL/Lockheed Missiles and Space Co.	Essentially, this is a study to determine the feasibility of assembly of an oscillator circuit within a glove-box system, using glove-box techniques. Assembly will be conducted in three separate gaseous atmospheres, ethylene oxide/Freon 12 mixture (ETO-F 12), sterile nitrogen and sterile air. Effects of ETO on soldering and potting operations, as well as the reliability of the test circuit, will be determined. Reliability of the microbiological integrity of the glove-box will also be recorded. Reviewed in JPL SPS No. 37-29, Vol IV, and 37-31, Vol IV.

Table 3-4. JPL Sterilization Group Tasks Recently Funded by NASA and Presently in the Contract Initiation Phase

Number	Subject	Description
1	Improvement of microbiological recovery techniques	A study to improve recovery techniques for recovering viable and sublethally damaged microorganisms. A physiological approach using combinations of incubation temperatures, oxygen tensions, pH, nutritional supplements, and light will be supplemented with the physical removal of microorganisms by rinse techniques containing surfactants and the newest drilling and impression techniques.
2	Microbiological examination of manufactured spacecraft parts	This task will use results from No. 1 above. Spacecraft parts may be subjected to drilling, grinding, sanding, cutting, fragmentation, pulverization, or dissolution in an inocuous solvent. Parts, or pieces thereof, will be placed into or on a suitable culture medium (media) and then incubated under optimum conditions. Evaluation of the data collected should give an indication of the level of contamination, type of parts contaminated, possible sources of contamination, and the levels at which adequate microbiological controls should be applied; i.e., before, during or after manufacture and/or assembly of parts into components, subsystems and systems.
3	Cleanroom prototype	This task will result in development of a cleanroom experimental laboratory. Monitoring techniques, cleanroom clothing and practices, and airflow patterns will be compared, evaluated and modified where indicated. This task constitutes one-half of the support for Project EASL.
4	Continuation of sterile assembly techniques study	This task will be a follow-on to the study described in Table 3-3, No. 4.
5	Training program development	This task will develop a training program covering all operations needed for the successful assembly, test and launch of a spacecraft that complies with the planetary quarantine requirement.

Table 3-5. JPL Sterilization Group Activities for Fiscal Year 1966

Number	Subject	Description
1	Review of heat sterilization and ETO specifications	This task would revise the present engineering specifications to reflect current knowledge of kill mechanisms of microorganisms based on the NASA-funded work of the USPHS, Cincinnati, Ohio (Drs. Angelotti and Reed) and MIT.
2	Physical and chemical methods of cleaning spacecraft parts	Survey of present sporicides, chemicals, detergents, and other cleaning compounds. Survey of microbiocidal matters such as lubricants and greases. Determine by microbiocidal experimentation the effectiveness of these materials. Recommend standard methods for reducing microbiological flora.
3	Automated techniques for microbiological sampling, recovery, and assay of microorganisms	Survey presently available and potentially useful automated equipment now being developed for bacteriological and chemical warfare and determine the applicability of these methods to problems of assay and certification of sterile spacecraft.

Table 3-6. Review of Research on Sterilizable Hardware

Title	JPL Cognizant Personnel	Expected Completion
Sterilizable parts (compatibility)	Lockyear	6-66
Polymeric materials screening (heat and ETO)	Harvey	6-66
Thermal control surfaces: problems defined	Hall/Kohorst	12-65
Sterilizable parachute materials	Nagler	12-64 (Comp)
Study of ratio of battery electrodes	Sweetnam/Uchiyama	2-65
Development of irradiated organic battery separators	Sweetnam/Uchiyama	8-65
Determine shelf life of Ag/Zn battery Cells	Sweetnam/Uchiyama	12-66
New organic separator materials	Sweetnam/Uchiyama	8-66
Study of electric propulsion attitude control actuators	Forney/Koch	12-65
Sterilizable antenna actuator	Forney/Koch	12-65
Sterilizable jet vane actuator	Forney/Koch	12-65
Sterilization test of Ranger/Mariner R midcourse shutoff accelerometer	Bachman/Hand	5-65
Test of first high-temperature image Dissection tube	Forney/Meisenholder	11-65
Image-dissection tube sterilization tests	Forney/Meisenholder	2-66
Evaluate results of sterilizable gas bearing gyro	Bachman/Lamb	5-65
Delivery of two sterilizable vidicons	Baker	6-66
Sterilizable Geiger-Müller tubes	Wengert	2-65
Scintillation crystals - life tests	Wengert	8-65
Optical cementing materials - final report	Wengert	3-65
Sterilizable photomultipliers	Wengert	2-65
Sterilizable solid state detectors	Wengert	6-65
Protection of sterilized biologic materials	Wengert	4-66
Sterilization of electrical connections; problems isolated	Goldsmith/Fitak	8-65
Electrical connections; follow-on work	Goldsmith/Fitak	6-66 (6-65)
Electrical connector tests; two types	Goldsmith/Fitak	11-66
Electronic fabrication process screening	Goldsmith/Fitak	9-65
Electronic fabrication processes; follow-on work	Goldsmith/Fitak	6-66 (6-65)
Capacitors - life test	Lockyear	8-66
Fixed resistors - life test	Lockyear	10-66
Variable resistors - life test	Lockyear	11-66

Table 3-6. Review of Research on Sterilizable Hardware (contd)

Title	JPL Cognizant Personnel	Expected Completion
Diodes, general - life test	Lockyear	8-67
Diodes, varactor - life test	Lockyear	1-66
Fuses - life test	Lockyear	9-66
Thermistors - life test	Lockyear	7-66
Transistors - life test	Lockyear	3-66
Relays - life test	Lockyear	10-66
Microcircuits, digital - life test	Lockyear	3-66
Inductors - life test	Lockyear	11-66
Microcircuits - linear	Lockyear	9-66
Temperature gradient tests - component parts	Lockyear	7-66
Life test follow-on's	Lockyear	FY66

C. MATHEMATICAL MODEL FOR COUNTING MICROBIAL CONTAMINATION ON THE SURFACE AND INTERIOR PARTS OF AN ASSEMBLY OF HARDWARE*

1. Purpose of the Model

a. This model has been developed so that: (1) a parametric evaluation of the variables which affect microbial contamination can be conducted, (2) an estimate of the microbial load can be generated with a relatively accurate confidence interval, and (3) a probabilistic model can be developed through conversion of this model into a density function.

b. Most of this subsection is written in mathematical terms, however, a narrative non-mathematical description is presented as follows:

(1) The process described by Figure 3-2 is the assembly of components into subassemblies, these into assemblies, assemblies into subsystems, and so on until all hardware is assembled into a final system assembly. This process of assembly affects total microbial load of the interior and exterior of the hardware assembly.

(a) The most obvious effect is that of having the microbial load on the surfaces increase due to contamination by the environment

* The effort to develop this model has only recently been initiated — the work shown is for general information and should be considered preliminary.

and by handling during assembly operations, storage, shipment, etc. (see Section IV. A. for a full discourse on microbial contamination).

(b) A second effect of the assembly operations is that of converting some or all of the microbial load from the exterior surface to the interior of the assembly. The interior microbial load is that portion which can only be killed by heat application; i.e., the interior microbial load can not be reduced by chemicals such as ETO since the microorganisms are buried in the interior of the hardware.

(2) The sequence depicted on Figure 3-3, is the process of assembling hardware and determining the total microbial load after considering the factors which reduce the load (the decontamination processes); the factors which increase the load (handling, environment, etc.); and distinguishing between the interior and exterior microbial load.

2. Description of Assembly Levels

Figure 3-2 depicts a general 5 level assembly process. The assembly levels shown (1 through 5) could portray the assembly of a module, with level 1 representing electronic piece-parts and subsequent levels representing chassis, nuts, bolts, etc.; or, it could portray the assembly of a capsule onto a spacecraft, with the levels 1 through 5 representing a capsule, spacecraft, attach hardware, OSE interface hardware, biological barriers, etc. Determination of assembly level (i level) is accomplished by determining the logic path requiring the maximum number of assembly levels (bubbles). Then the operation is reversed and all bubbles are numbered decreasingly from its predecessor. The assembly level numbers are assigned in this way only for the purpose of simplifying the mathematical summation.

3. Description of Symbols

a. I = Microbial contamination of the assembly - interior. Range of I: $I \geq 0$.

Interior is defined as any location which cannot, with 100 percent effect, be penetrated by gaseous ETO. All microorganisms in interiors will remain viable after the assembly has been exposed to ETO.

b. E = Microbial load contaminating the exterior of the assembly. Range of E: $E \geq 0$.

Exterior is defined to include all locations not meeting the definition of I above. Microorganisms which are on these surfaces will be fully subjected to the kill affect of an ETO application.

c. K = the survival factor operating on I for whatever decontamination process is being used. It is a function of assembly size and shape, material, heat transfer, temperature, temperature come up time, and time at temperature. For high temperature over extended time K = 0 and the decontamination cycle is a sterilization cycle. For an ETO application at normal temperature K = 1 and the decontamination cycle does not reduce the interior contamination. Range of K: $0 \leq K \leq 1$.

d. k = the survival factor operating on E for whatever decontamination process is being used. It is a function of assembly size and shape, materials, temperature, come up time, time at temperature, humidity, and ETO concentration. When the hardware is being subjected to a dry heat cycle $k \leq K$. The difference will depend upon the relative heat levels on the interior versus exterior, humidity, and whether or not ETO was used (this merely states that a given dry heat cycle may kill more on the surface than inside the assembly). Range of k: $0 \leq k \leq 1$.

e. Contamination function

a) α = the exterior microbial contamination collected on the assembly after the decontamination cycle and through completion of next level of assembly. This will include contamination due to handling, storage, testing, and transportation. Probably the most significant contaminating attribute of α is the handling required during the assembly operation. Range of α: $\alpha \geq 0$.

b) β = the exterior microbial contamination collected on the assembly after completion of the assembly operation and immediately prior to the next decontamination cycle. This includes all of the factors listed for α except actual assembly operations. Range of β: $\beta \geq 0$.

f. μ = the assembly operator which represents the percentage of E, β, or α microorganisms which are converted to I values due to the assembly operation e.g., hermetic seal, potting, bolted plates, etc.

(1) $\mu = 1$ when the assembled unit seals all the microorganisms inside the assembly such that these microorganisms now satisfy the definition for I given in 3. a. above.

(2) $\mu = 0$ when the assembled unit seals none of the E, β, or α microorganism inside the assembly; i.e., E, β, and α all satisfy 3. b. above after assembly.

(3) $0 < \mu < 1$ for all other conditions of assembly.

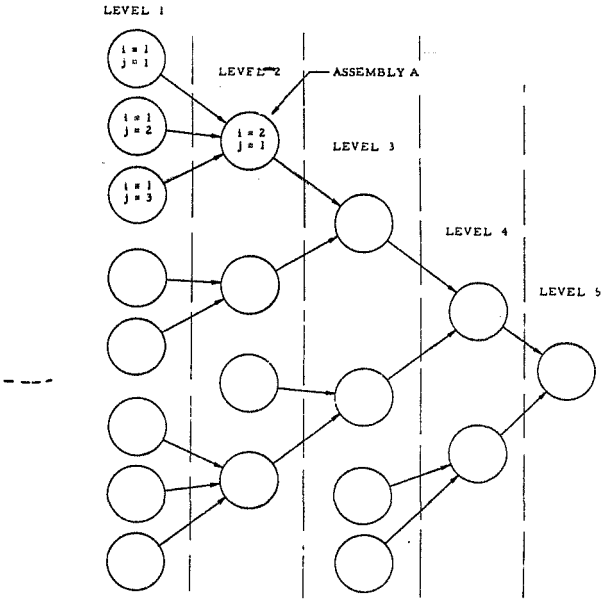


Figure 3-2. Logic Diagram No. 1

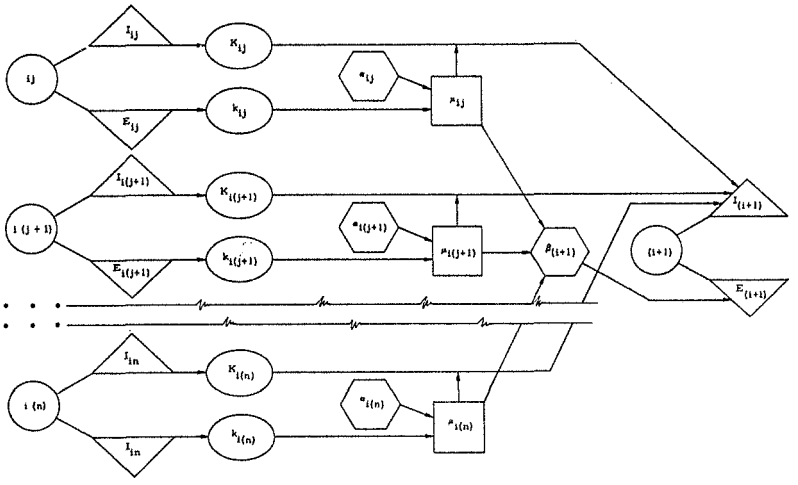
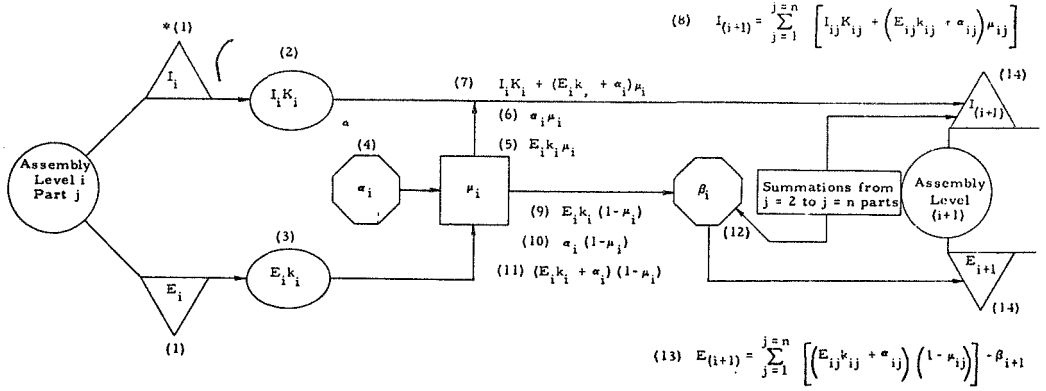


Figure 3-3. Logic Diagram No. 2



* Numbers in ( ) correspond to steps outlined in Paragraph C. 4. a.

Figure 3-4. Logic Diagram No. 3

4. First Order Summation

The following is a typical example of the steps required to perform the microbial count on the (i + 1) level assembly.

a. Perform the following on each path leading from the i level to (i + 1) level (see Figs. 3-3 and 3-4).

(1) Determine I_i and E_i values (computed from assay data – see Section IV).

(2) Compute I_iK_i, the I value after the decontamination cycle.

(3) Compute E_ik_i, the E value after the decontamination cycle.

(4) Determine α_i.

(5) Compute $E_ik_i\mu_i$, the incremental I value added to interior contamination as a result of the assembly operations.

(6) Compute $\alpha_i\mu_i$, the incremental I value added to interior contamination by α_i as a result of the assembly operations.

(7) Compute $I_iK_i + E_ik_i\mu_i + \alpha_i\mu_i = I_iK_i + \left(E_ik_i + \alpha_i\right)\mu_i$, the total internal contamination from one of the parts being assembled.

(8) Compute $I_{(i+1)}$ the total internal contamination from all parts being assembled,

$$I_{(i+1)} = \sum_{j=1}^{j=n} \left[I_{ij}K_{ij} + \left(E_{ij}k_{ij} + \alpha_{ij}\right)\mu_{ij} \right]$$

where j is the symbol assigned to each part being assembled and n is the total number of parts in the (i + 1) assembly.

(9) Compute $E_ik_i\left(1 - \mu_i\right)$, the incremental E_i value remaining in E state after decontamination cycle and the assembly operation.

(10) Compute $\alpha_i\left(1 - \mu_i\right)$, the incremental α_i value remaining in E state after the assembly operation.

(11) Compute $E_ik_i\left(1 - \mu_i\right) + \alpha_i\left(1 - \mu_i\right) = \left(E_ik_i + \alpha_i\right)\left(1 - \mu_i\right)$, the external contamination due to one of the parts being assembled.

(12) Determine $\beta_{(i+1)}$.

(13) Compute $E_{(i+1)}$, the total external contamination from all parts being assembled,

$$E_{(i+1)} = \beta_{(i+1)} + \sum_{j=1}^{j=n} \left[\left(E_{ij} k_{ij} + \alpha_{ij} \right) \left(1 - \mu_{ij} \right) \right]$$

b. The following is a narrative example of the above steps — the appropriate steps are noted in [Brackets].

1) From Figure 3-2 the contamination load for assembly A is to be computed. This assembly is i = 2 and there are n = 3 parts being assembled.

2) Select parts j = 1, 2 and 3 and determine from assay computation the interior and exterior microbial load [1].

3) Subject the parts to a decontamination cycle and determine the interior and exterior microbial load surviving the cycle [2 and 3].

4) Through a system of monitoring, estimate the microbial load added to each part during handling and assembly [4].

5) Through analysis of engineering data and assembly procedures, compute the percentage of exterior microbial load on each part which is converted to interior microbial load when assembled, and compute the microbial load remaining on the exterior [5, 6, 7, 9, 10 and 11].

6) Through a system of monitoring similar to that in step (4) estimate the amount of surface microbial load added after completion of assembly and prior to the next decontamination cycle [12].

7) Sum the exterior and interior microbial loads computed in steps 5) and 6) to determine the net microbial contamination [8, 13].

5. Total Summation

This is to be the mathematical expression for the total summation of all levels of assembly from i = 1 to i = m; i.e., m levels of assembly. Figure 3-2 is the assembly logic in which m = 5 for 5 levels of assembly